African Journal of Biotechnology Vol. 10(64), pp. 14029-14037, 19 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.539
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Role of plant growth regulators in preservation of *Pyrus* germplasm *in vitro*

Maqsood Ahmed[1]*, Muhammad Akbar Anjum[2], Mohammad Jamil Ahmed[1], Ghulam Mustafa Sajid[3], Abid Yoqub[1] and Muhammad Shafqat[1]

[1]Department of Horticulture, University of Azad Jammu and Kashmir, Faculty of Agriculture, Rawalakot, State of the Azad Jammu and Kashmir, Pakistan.
[2]University College of Agriculture, Bahauddin Zakariya University, Multan, Pakistan.
[3]Plant Genetic Resources Programme, National Agricultural Research Centre (NARC), Islamabad, Pakistan.

Accepted 5 August, 2011

***In vitro* established shoots of nine pear genotypes namely; Khurolli, Bagugosha, Pathar nakh, Desi nakh, Kotharnul, Btangi, Frashishi, Kashmiri nakh, and Raj btung, were preserved by lowering concentration of growth regulators [(0.00, 0.25 or 0.50mg l^{-1} 6-benzyaminopurine (BAP) or adding growth retardants, 10 mg L^{-1} Alar (diaminazide or B-9), or abscisic acid (ABA)] in the culture medium. The cultures were assessed for their survival and regenerability percentages after 3, 6, 9 and 12 months of storage. The genotypes differed significantly for survival and regeneration percentage. The culture medium supplemented with 0.50 mg L^{-1} BAP was comparatively more effective and resulted in higher survival rate and re-growth after transferring onto the fresh medium. Storage for short duration (3 months) resulted in significantly higher survival and regeneration rates than other storage periods. As storage period was prolonged, survival and regenerability of shoots progressively decreased. Overall results indicated that the shoots of Desi nakh had the highest survival (73.33 %) and Pathar nakh showed the maximum regenerability (67.66 %) when cultured on the medium supplemented with 0.50 mg L^{-1} BAP and stored for 3 months. However, no shoots of Kotharnul survived when cultured on the medium without any growth regulator and kept for 12 months.**

Key words: Growth retardants, *in vitro* preservation, pear genotypes, slow growth.

INTRODUCTION

Plant genetic resources in fruit crops and their variability in the form of wild relatives are of immense value to mankind. Plant breeders also require reservoir of genetic variation for crop improvement. Genetic diversity in fruit trees is being eroded and under threat due to many factors like deforestation, urbanization, introduction of new cultivars, natural calamities and adverse ecological conditions. Fruit trees in Central Asia are also threatened by logging and fuel wood gathering (Whigham et al., 1993).

Pyrus germplasm in the region of Azad Jammu and Kashmir (Northern Pakistan) presents rich diversity among fruit species as a result of hybridization, mutation and naturally seed based propagation. Wild populations of *Pyrus* species are threatened worldwide, and even many pear varieties were lost in the 19th century, continuing even today (Fowler and Mooney, 1990; Endtmann, 1999; Sindelar, 2002). Wunsch and Hormaza (2007) revealed that a low level of variability in cultivated pear genotypes is alarming and hence there is need to widen the genetic base of pear germplasm and conserve an adequate level of gene pool for future use in pear breeding programmes. The process of genetic erosion in *Pyrus* germplasm has been seriously realized and attempts have been made to conserve the germplasm by *in situ* and *ex situ* preservations (Wagner, 1999; Paprstein et al., 2002). Although, diversity in fruit tree germplasm is usually conserved under field conditions (field gene bank or orchards), traditional methods are expensive due to high labour cost, the large space required and vulnerable to environmental hazards.

Furthermore, these field gene banks do not represent the entire range of genetic variability within the respective genus. This led to the consideration of *in vitro* techniques

*Corresponding author. E-mail: msqahmed@yahoo.com.

and cryopreservation for germplasm conservation (Withers and Alderson, 1986). *In vitro* culture system has high degree of genetic stability and offers disease-free source of plant multiplication.

Slow growth and cryopreservation methods are widely used to maintain germplasm collections *in vitro* (Scowcroft, 1984). After preservation through these techniques, cultures can be readily brought back to normal culture conditions at any time to produce plants on desire. Minimal growth methods for preservation of fruit plants are well established (Wilkins and Dodds, 1983; Wilkins et al., 1985). Therefore, *in vitro* shoot tip culture under minimal growth storage conditions (also called slow growth storage or growth suppression) represents a reliable mean of fruit tree conservation. This method is relatively convenient and economical for maintaining large number of genotypes and considered as immediate alternate solution for short to medium term storage of fruit germplasm. *In vitro* culture is an effective method for *ex situ* conservation of plant genetic diversity (Fay, 1994), allowing rapid multiplication with genetically stable plantlets (Rao, 2004). The *in vitro* minimal growth preservation can be achieved by lowering incubating temperature, modifying / manipulating culture medium (altered nutrient availability) or supplementing with osmotically active compound like mannitol. In addition to these, reducing concentration of growth regulators and adding growth retardants such as Alar or B-9 and abscisic acid (ABA) are also useful to suppress shoot growth and lengthen subculture duration at normal culture temperatures. This storage technique is generally applicable to wide range of fruit tree genotypes in extending the ordinary subculture duration from few weeks to 6 months. Plant species have already been preserved by reducing concentration of growth regulators or omitting cytokinins and adding growth retardants such as Alar (diaminazide or B-9), maleic hydrazide and chlorocholine chloride (CCC) (Westcott, 1981; Gunning and Lagerstedt, 1985) at normal temperature. These alternate preservation techniques are less costly and safe to conserve germplasm (Epperson et al., 1997). Minimal growth storage is a very simple technique and has been studied in several laboratories for *Pyrus* germplasm conservation (Wanas et al., 1986; Wilkins et al., 1988; Moriguchi et al., 1990; Moriguchi, 1995; Ahmed and Anjum, 2010; Ahmed et al., 2010). Depending on species, these stored plants can be micropropagated rapidly when desired.

Keeping in view the importance of existing genetic diversity in *Pyrus* germplasm in Azad Jammu and Kashmir, attempts were made to develop a slow growth storage technique by using reduced concentration of cytokinin or addition of a growth retardant in culture medium to conserve the germplasm. The aim of this study was to extend subculture duration and develop a suitable storage technique for short to medium term preservation of divergent pear genotypes.

MATERIALS AND METHODS

Bud wood of nine genotypes of pear namely; Khurolli, Bagugosha, Pathar nakh, Desi nakh, Kotharnul, Btangi, Frashishi, Kashmiri nakh, and Raj btung, were collected from different areas of Azad Jammu and Kashmir and grafted onto wild rootstock (Btangi) in the nursery at National Agricultural Research Centre (NARC) Islamabad. Shoot tips of these genotypes were excised from the plants, surface sterilized and established on MS medium (Murashige and Skoog, 1962) supplemented with 30 g L^{-1} sucrose, 7 g L^{-1} agar and 1 mg L^{-1} BAP. From established *in vitro* cultures, shoots tips of about 1 to 1.5 cm long of these genotypes were excised aseptically and placed immediately in 200 ml culture jars containing 30 ml of culture medium (MS medium supplemented with lower concentration of growth regulators at 0.00, 0.25 or 0.50 mg L^{-1} BAP, or with a growth retardant, Alar or ABA, at 10 mg L^{-1} for preservation. The culture jars were covered with their lids and kept at 25 ± 2°C in 16 h photoperiod under white fluorescent light intensity of 55 µmol m^{-2} s^{-1} for different periods (3, 6, 9, and 12 months). The experiment was factorial in completely randomized design (CRD) consisting of nine genotypes, five culture media and four storage periods with three replications. There were 10 shoots in each replication. Data were recorded on survival of the shoots after every storage period and for regenerability after one month of transferring on to the fresh culture medium.

Assessment of cultures

Survival of the cultures was assessed on the basis of criteria as suggested by Reed (1992): dead and brown shoots were considered as unsurvived, while those with vigorous growth and having healthy leaves were considered as survived.

$$\text{Survival percentage} = \frac{\text{Number of shoots survived}}{\text{Number of shoots transferred}} \times 100$$

For regenerability of cultures, shoots were removed from the cultures, trimmed off to a length of 1 to 1.5 cm without damage and transferred into fresh shoot proliferation medium (MS medium supplemented with 30 g L^{-1} sucrose, 7 g L^{-1} agar and 1 mg L^{-1} BAP). The cultures were kept at 25 ± 2°C in 16 h photoperiod under white fluorescent light intensity of 55 µmol m^{-2} s^{-1}. The data regarding regeneration percentage were recorded after one month on the basis of growth initiation by using the following formula.

$$\text{Regeneration percentage} = \frac{\text{Number of shoots regenerated / revived growth}}{\text{Number of shoots transferred on culture medium}} \times 100$$

Statistical analysis

Data collected were subjected to statistical analysis based on analysis of variance method. Means obtained were compared by employing Duncan's multiple range (DMR) test at 5% probability using MSTAT-C statistical computer package (Michigan State University, East Lancing, MI).

RESULTS

Survival and regenerability of shoots of the nine pear genotypes was assessed as affected by lower concen-

Table 1. Survival percentage of cultures as affected by growth factors, storage periods and pear genotypes.

(a) Pear genotype		(b) Growth factor	
Factor/Treatment	**Survival (%)**	**Factor/Treatment**	**Survival (%)**
Khurolli	32.70[a]	T_0 (control)	15.95[d]
Bagugosha	33.72[a]	T_1 (0.25 mg/ L BAP)	22.87[c]
Pathar nakh	28.72[ab]	T_2 (0.50 mg/ L BAP)	39.19[a]
Desi nakh	28.70[ab]	T_3 (10 mg/ L alar)	29.47[b]
Kotharnul	22.33[c]	T_4 (10 mg/ LABA)	23.64[c]
Btangi	24.00[bc]	**(c) Storage period**	
Frashishi	23.08[c]	3 months	39.73[a]
Kashmiri nakh	22.17[c]	6 months	31.27[b]
Raj btung	20.97[c]	9 months	21.51[c]
-	-	12 months	12.31[d]

*Means sharing similar letter(s) in a group are non-significant at α = 5% (DMR test).

tration of growth regulators (BAP) or by adding a growth retardant (Alar or ABA) in the medium, stored for 3, 6, 9 and 12 months. The results were compared to determine the best growth factors for storage of genetically diverse pear collections.

The results indicate that the genotypes differed significantly for the survival and regenerability after transferring into the fresh medium. The highest survival percentage was recorded in the shoots of Bagugosha followed by those of Khurolli, Pathar nakh and Desi nakh (Table 1), while the highest regeneration percentage was found in the shoots of Bagugosha followed by Khurolli and Pathar nakh (Table 3). The shoots of Raj btung, Kashmiri nakh, Kotharnul, Frashishi and Btangi had significantly low values for both the parameters as compared to other genotypes.

Significant differences were also found among the treatments of growth factors in the culture medium. The maximum survival and regenerability percentages were recorded in those shoots which were cultured on the medium containing 0.5 mg L^{-1} BAP and this significantly differed from other media (Tables 1 and 3). Poor performance with the lowest percentage of survival and re-growth was obtained in the shoots cultured on the medium without any growth factor (growth regulator or growth retardants).

As far as storage duration is concerned, all the storage periods differed significantly from each other. As the storage period was increased, survival and regeneration percentages of the cultures progressively decreased. The maximum survival and regeneration percentages were recorded in the shoots stored for 3 months, whereas, the minimum percentages for both the parameters were observed in the shoot cultures which were stored for 12 months (Tables 1 and 3).

The mean values for interaction between growth factor treatments and storage periods revealed that the highest survival and regeneration percentages were observed in the shoots cultured on the medium supplemented with 0.5 mg L^{-1} BAP for 3 months. This treatment combination was significantly superior to all other treatment combinations. Whereas, the lowest survival and regenerability was recorded in the shoots cultured on medium without any growth factor and on the medium containing 0.25 mg L^{-1} BAP both stored for 12 months (Figures 1a and 2a).

Regarding interaction between genotypes and growth factors treatments, same trend was observed for survival percentage and regeneration percentage, which revealed that the shoots of Pathar nakh cultured on medium supplemented with 0.5 mg L^{-1} BAP exhibited the highest survival during storage and regenerability when transferred into the fresh medium, followed by the shoots of Bagugosha and Khurolli also cultured on the same medium. The lowest survival and regeneration percentages were recorded in the shoots of Kotharnul, cultured on the medium without any growth factor (Figures 1b and 2b).

Combined effect of genotypes and storage duration indicated that the highest survival and regeneration percentages were recorded in the shoots of Desi nakh stored for 3 months, followed by those of Bagugosha for the same period of storage. The minimum rates of survival and regenerability were achieved in the shoots of Frashishi stored for 12 months (Figures 1c and 2c). The three-way interaction among the three factors (genotypes, growth factors and storage periods) indicated that the maximum survival percentage was recorded in the shoots of Desi nakh, and highest regenerability in the shoots of Pathar nakh cultured on the medium supplemented with 0.5 mg L^{-1} BAP and stored for 3 months. However, all the shoots of Kotharnul died when cultured on medium without any growth factor and stored for 12 months. In addition, shoots of Pathar nakh and Btangi also responded poorly when cultured on the same medium for the same storage period (Tables 2 and 4).

Table 2. Survival percentage of pear genotypes as affected by the interaction among growth factors, storage periods and pear genotypes.

Storage period	Khurolli	Bagugosha	Pathar nakh	Desi nakh	Kotharnul	Btangi	Frashishi	Kashmiri nakh	Raj btung
T_0 (control)									
3 months	32.00^{i-z}	15.33^{xyz}	18.00^{u-z}	50.66^{a-p}	15.00^{a-f}	16.00^{w-z}	16.33^{w-z}	17.00^{u-z}	16.66^{s-z}
6 months	32.33^{h-z}	22.33^{q-z}	17.66^{u-z}	9.66^{a-f}	7.00^{a-f}	34.00^{f-z}	20.66^{s-z}	34.00^{f-z}	11.00^{w-z}
9 months	13.33^{yz}	22.33^{q-z}	7.00^{a-f}	9.00^{a-f}	5.33^{a-f}	7.33^{a-f}	33.00^{j-z}	34.00^{f-z}	18.00^{r-z}
12 months	6.33^{a-f}	11.00^{z}	1.33^{ef}	5.00^{a-f}	0.00^{f}	2.33^{def}	3.66^{c-f}	5.00^{a-f}	8.00^{z}
T_1 (0.25 mg L^{-1} BAP)									
3 months	36.66^{e-z}	48.66^{a-s}	38.00^{e-z}	52.66^{a-p}	43.00^{c-x}	15.00^{xyz}	21.33^{r-z}	36.66^{e-z}	36.66^{d-y}
6 months	39.00^{d-z}	47.00^{a-t}	22.00^{q-z}	17.00^{u-z}	14.00^{yz}	31.00^{i-z}	34.00^{f-z}	16.33^{w-z}	29.33^{g-z}
9 months	16.66^{v-z}	15.00^{xyz}	17.00^{u-z}	28.00^{l-z}	6.66^{a-f}	33.33^{g-z}	7.66^{a-f}	15.00^{xyz}	9.66^{xyz}
12 months	18.66^{u-z}	16.00^{w-z}	9.33^{a-f}	9.00^{a-f}	13.66^{yz}	8.66^{a-f}	8.00^{a-f}	6.66^{a-f}	6.00^{ab}
T_2 (0.50 mg L^{-1} BAP)									
3 months	68.00^{abc}	61.66^{a-f}	71.33^{ab}	73.33^{a}	57.00^{a-j}	50.00^{a-q}	41.00^{c-y}	54.00^{a-q}	36.66^{d-y}
6 months	50.66^{a-p}	53.33^{a-p}	56.33^{a-k}	51.66^{a-p}	55.33^{a-l}	58.33^{a-i}	53.33^{a-q}	36.66^{e-z}	31.00^{f-z}
9 months	44.66^{b-v}	55.00^{a-m}	61.00^{a-g}	30.33^{j-z}	27.00^{m-z}	25.66^{p-z}	15.33^{xyz}	7.33^{z}	30.00^{g-z}
12 months	17.66^{u-z}	21.00^{s-z}	35.33^{f-z}	20.66^{s-z}	16.00^{w-z}	8.66^{a-f}	13.33^{yz}	18.33^{r-z}	7.66^{z}
T_3 (10 mg L^{-1} Alar)									
3 months	63.66^{a-e}	65.66^{a-d}	60.00^{a-h}	39.66^{s-z}	36.66^{e-z}	31.33^{i-z}	43.66^{c-w}	41.66^{c-u}	32.66^{e-z}
6 months	54.33^{a-n}	54.33^{a-n}	36.66^{e-z}	31.00^{i-z}	14.33^{yz}	36.66^{e-z}	17.33^{u-z}	31.66^{f-z}	31.00^{f-z}
9 months	30.00^{j-z}	18.66^{u-z}	33.66^{e-z}	26.00^{o-z}	38.00^{e-z}	21.66^{r-z}	10.00^{a-f}	8.00^{z}	19.00^{r-z}
12 months	31.33^{g-z}	13.33^{yz}	17.66^{u-z}	13.66^{yz}	12.66^{yz}	21.33^{r-z}	9.33^{a-f}	5.00^{ab}	9.33^{yz}
T_4 (10 mg L^{-1} ABA)									
3 months	33.33^{g-z}	57.33^{a-j}	19.66^{t-z}	49.66^{a-q}	45.00^{b-u}	32.66^{h-z}	49.33^{a-r}	32.33^{e-z}	15.00^{t-z}
6 months	36.33^{e-z}	18.33^{u-z}	16.66^{v-z}	30.00^{j-z}	14.66^{xyz}	17.00^{u-z}	33.33^{j-z}	23.66^{n-z}	31.33^{f-z}
9 months	12.66^{yz}	28.66^{k-z}	17.00^{u-z}	17.66^{u-z}	18.33^{u-z}	22.00^{q-z}	26.66^{n-z}	11.66^{w-z}	17.00^{r-z}
12 months	16.33^{w-z}	29.33^{j-z}	18.66^{u-z}	9.33^{a-f}	7.00^{a-f}	7.00^{a-f}	4.33^{b-f}	8.33^{yz}	23.33^{o-z}

*Means sharing similar letter(s) are non-significant at α = 5% (DMR test).

DISCUSSION

Growth rate of *in vitro* cultures can be reduced and time of subculture prolonged by altering the growth factor in culture medium (decreasing the concentrations of growth regulators or adding growth retardants). The technique was employed for short term *in vitro* preservation of shoot tips of nine pear genotypes in the present study. The genotypes differed for their survival and regeneration percentages in response to change in growth factor. Performance of the genotypes

Table 3. Regeneration percentage of cultures as affected by growth factors, storage periods and pear genotypes.

(a) Pear genotype		(b) Growth factor	
Factor/Treatment	**Survival (%)**	**Factor/Treatment**	**Survival (%)**
Khurolli	29.73^{ab}	T_0 (control)	14.44^{d}
Bagugosha	31.87^{a}	T_1 (0.25 mg/ L BAP)	21.35^{c}
Pathar nakh	27.13^{abc}	T_2 (0.50 mg/ L BAP)	37.25^{a}
Desi nakh	26.57^{bc}	T_3 (10 mg/ L Alar)	27.64^{b}
Kotharnul	20.12^{d}	T_4 (10 mg/ L ABA)	22.16^{c}
Btangi	22.22^{cd}	**(c) Storage period**	
Frashishi	21.92^{d}	3 months	37.61^{a}
Kashmiri nakh	21.43^{d}	6 months	29.61^{b}
Raj btung	20.13^{d}	9 months	19.86^{c}
-	-	12 months	11.01^{d}

*Means sharing similar letter(s) in a group are non-significant at α = 5% (DMR test).

Pathar nakh, Desi nakh, Khurolli and Bagugosha was comparatively better than other genotypes. This variation among the genotypes might be due to their genotypic behaviour or endogenous levels of growth regulators. Similar observations were reported by Wilkins et al. (1988), who reported that *in vitro* preservation of woody tree cultures was dependent on techniques and genotypes.

Results indicate that the genotypes require an optimum level of exogenously growth regulator(s) for growth of their cultures. If the level of growth regulator(s) is decreased, the growth rate of the cultures is reduced. Similarly, addition of growth retardants also suppresses the growth of cultures. Reduced concentration of growth regulator(s) results in slow process of senescence (Wilkins et al., 1988). In the present study, using low concentration of cytokinin (BAP) at 0.5 mg L^{-1} remained successful with slow process of senescence, showing high rates of survival and regenerability. However, the culture medium without any growth factor (growth regulator free, control medium) resulted in minimum survival and regeneration percentages. This indicates that *in vitro* cultured shoots have necessary requirements for supply of growth regulator(s) for their survival and maintaining the potential for initiation of new growth after subculturing.

Although, growth retardants have been successfully used for *in vitro* preservation of different plant species, yet ABA acts as natural growth retardant and controls dormancy in potato (Addicott and Lyon, 1969). Storage of plantlets of two apple genotypes with the addition of ABA when stored in jars and tubes, improved the condition of cultures stored in tubes and decreased viability in jars (Kovalchuk et al., 2009). Addition of growth retardant (Alar or ABA) in culture medium resulted in reduced survival and regeneration rates. Survival and regeneration percentages were comparatively higher when Alar (10 mg L^{-1}) was added to the medium than when ABA was included. Negri et al. (2000) found that low BAP and ABA resulted in the poorest storage of two apple genotypes while moderate BAP with or without ABA was successful for longer storage. The average survival rate and regenerability of cultures remained less than 30 percent in both cases. Wilkins et al. (1988) used different techniques for *in vitro* preservation of apple scion variety 'Greensleeves' and other woody trees, including reduced culture temperatures, modification in medium and addition of growth retardants in culture medium. They concluded that the retardants were ineffective to suppress growth for *in vitro* germplasm storage of woody species. On the other hand, Westcott et al. (1977) found that interval of subculturing in potato tissue cultures could be extended by adding ABA to the culture medium. They obtained enhanced survival at all concentration levels but the most successful results were obtained when 5 and 10 mg L^{-1} ABA was added to the medium. These results indicate that the type and concentration of growth retardants to suppress the growth of cultures depends upon the endogenous levels of growth regulators in explants and nature of plant species.

Reduced concentration of a cytokinin (0.25 or 0.50 mg L^{-1} BAP) in the culture medium maintained survival and regeneration rate after short term storage. However, growth regulator free culture medium resulted in minimum survival and regeneration percentages, obviously showing deficiency and marked effect on growth rate with passage of storage periods. This indicates that absence of cytokinin corresponded to sharp reduction in growth of the cultures and lost the potential for resuming new growth after transferring onto fresh culture medium. As far as the growth retarding compounds

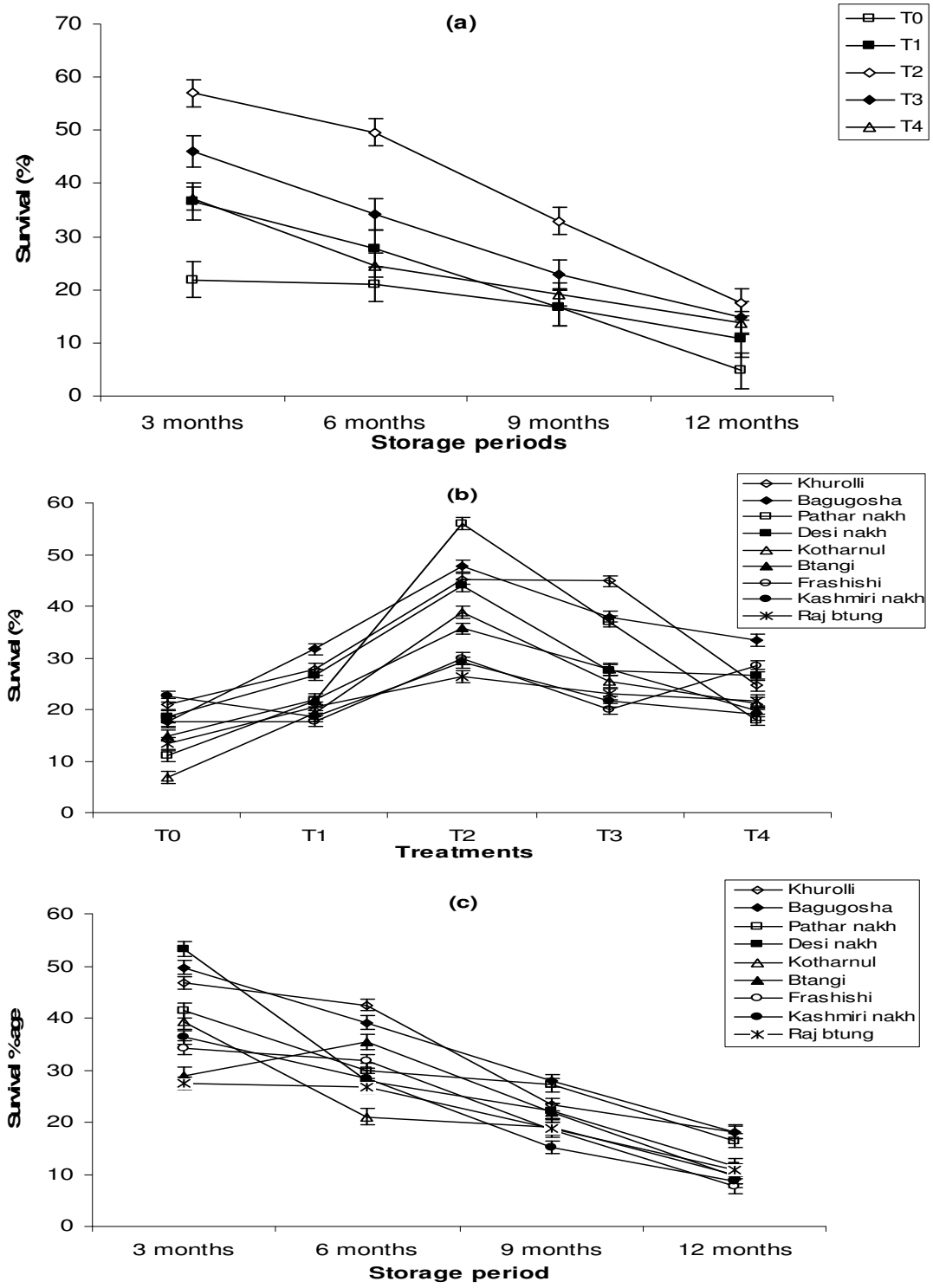

Figure 1. Effect of interaction between various factors on survival percentage of cultures; a) between growth factor treatments and storage periods, b) between genotypes and growth factor treatments and c) between genotypes and storage periods.

are concerned, overall growth rate of the cultures were slowed down and the time of subculturing prolonged, but Alar was comparatively more effective for both parameters. However, presence of growth retardants for longer period of time might have pronounced physiological effects; possibly create resistance or tolerance to growth retardant. Such types of shoots would survive better and initiate physiologically normal growth

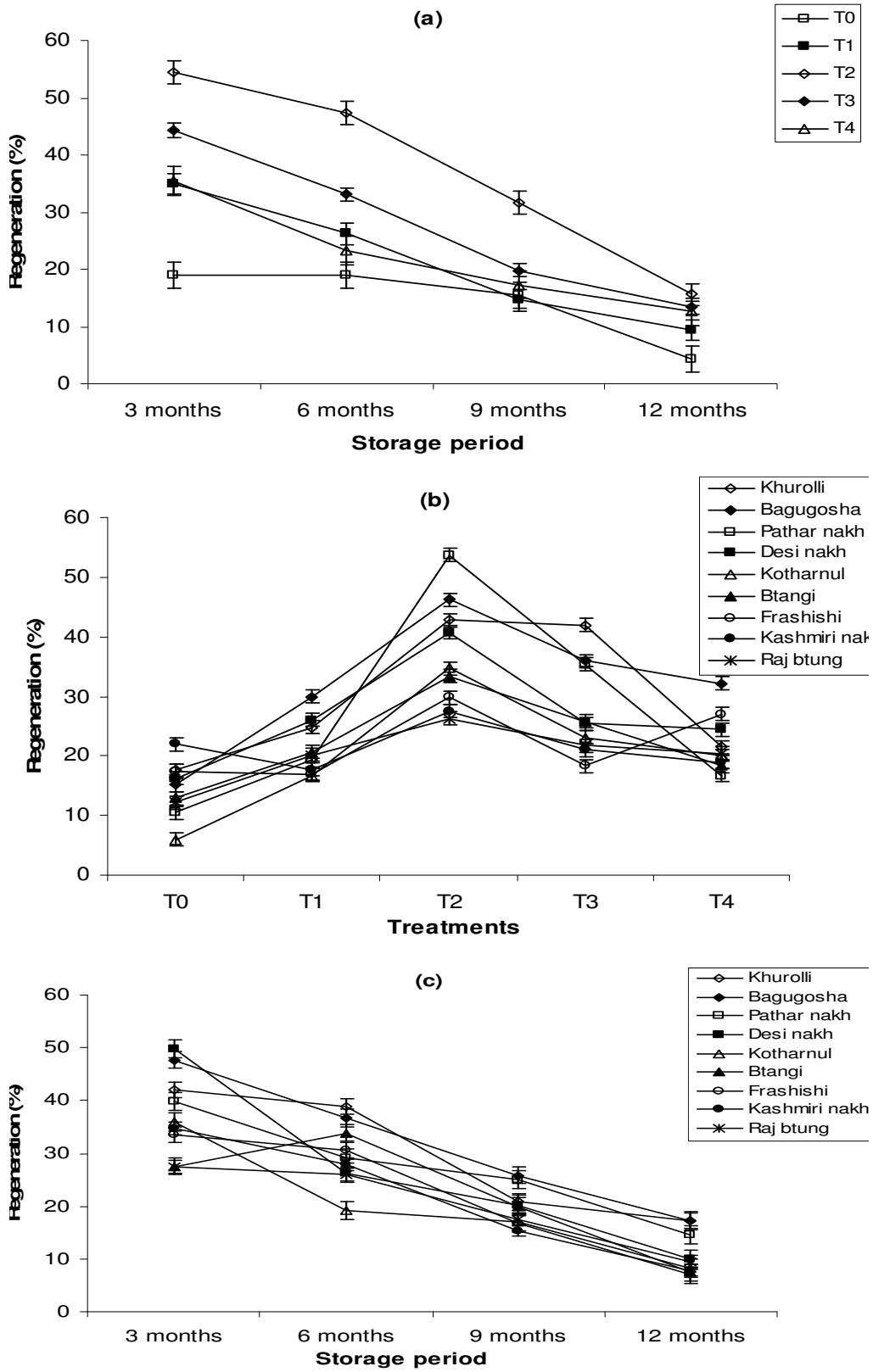

Figure 2. Effect of interaction between various factors on regeneration percentage of cultures; a) between growth factor treatments and storage periods, b) between genotypes and growth factor treatments and c) between genotypes and storage periods.

Table 4. Regeneration percentage of pear genotypes as affected by the interaction among growth factors, storage periods and pear genotypes.

Storage period	Khurolli	Bagugosha	Pathar nakh	Desi nakh	Kotharnul	Btangi	Frashishi	Kashmiri nakh	Raj btung
T_0 (control)									
3 months	25.00^{k-z}	12.66^{w-z}	17.33^{t-z}	45.66^{a-s}	13.00^{w-z}	13.00^{w-z}	15.00^{t-z}	14.33^{t-z}	14.66^{t-z}
6 months	27.66^{i-z}	17.66^{t-z}	18.66^{s-z}	7.66^{a-h}	7.00^{a-h}	31.33^{h-z}	19.33^{r-z}	32.00^{g-z}	10.33^{yz}
9 months	11.00^{yz}	20.33^{p-z}	5.00^{c-h}	7.33^{a-h}	4.00^{e-h}	6.33^{a-h}	32.33^{g-z}	36.33^{c-z}	17.00^{t-z}
12 months	6.00^{a-h}	9.66^{z}	1.00^{gh}	4.33^{d-h}	0.00^{h}	1.00^{gh}	3.33^{fgh}	5.33^{b-h}	6.66^{a-h}
T_1 (0.25 mg L^{-1} BAP)									
3 months	33.00^{f-z}	47.00^{a-q}	35.66^{d-z}	52.33^{a-k}	39.00^{b-x}	13.66^{v-z}	20.00^{p-z}	36.33^{c-z}	37.33^{b-z}
6 months	34.33^{f-z}	45.33^{a-s}	19.66^{q-z}	17.33^{t-z}	10.66^{y-z}	31.33^{h-z}	34.00^{f-z}	14.00^{u-z}	29.33^{h-z}
9 months	14.00^{u-z}	12.00^{w-z}	14.33^{t-z}	26.00^{j-z}	5.33^{b-h}	30.66^{h-z}	7.33^{a-h}	14.66^{t-z}	9.33^{a-h}
12 months	17.66^{t-z}	15.33^{t-z}	7.66^{a-h}	8.33^{a-h}	11.66^{w-z}	7.00^{a-h}	6.33^{a-h}	6.00^{a-h}	4.66^{c-h}
T_2 (0.50 mg L^{-1} BAP)									
3 months	64.00^{ab}	59.00^{a-g}	67.66^{a}	66.33^{a}	52.00^{a-k}	48.00^{a-o}	41.00^{a-v}	52.33 $^{a-k}$	39.33^{b-w}
6 months	46.66^{a-r}	49.33^{a-n}	53.33^{a-j}	50.66^{a-m}	53.00^{a-n}	56.66^{a-h}	52.33^{a-n}	35.00^{e-z}	30.00^{h-z}
9 months	45.33^{a-s}	55.00^{a-i}	62.33^{a-d}	27.00^{j-z}	22.66^{n-z}	23.00^{n-z}	13.33^{v-z}	6.33^{a-h}	29.00^{h-z}
12 months	15.33^{t-z}	21.33^{o-z}	31.66^{h-z}	19.00^{s-z}	11.33^{xyz}	6.00 $^{a-h}$	12.66^{w-z}	16.33^{t-z}	6.66^{a-h}
T_3 (10 mg L^{-1} Alar)									
3 months	62.00^{a-e}	63.00^{abc}	59.66^{a-f}	37.33^{b-z}	33.66^{f-z}	29.66^{h-z}	41.66^{a-u}	39.33^{b-w}	33.00^{f-z}
6 months	52.00^{a-k}	51.66^{a-l}	37.66^{b-y}	28.66^{i-z}	12.33^{w-z}	36.00^{c-z}	15.66^{t-z}	34.33^{f-z}	29.33^{h-z}
9 months	23.33^{m-z}	17.33^{t-z}	28.00^{i-z}	25.00^{k-z}	34.33^{f-z}	19.33^{r-z}	7.66^{a-h}	7.00^{a-h}	16.33^{t-z}
12 months	30.66^{h-z}	12.00^{w-z}	16.00^{t-z}	10.66^{yz}	12.00^{w-z}	18.33^{s-z}	8.33^{a-h}	3.33^{fgh}	8.33^{a-h}
T_4 (10 mg L^{-1} ABA)									
3 months	26.33^{j-z}	56.66^{a-h}	18.66^{s-z}	47.33^{a-p}	42.00^{a-t}	33.33 $^{f-z}$	49.66^{a-n}	31.33^{h-z}	13.33^{v-z}
6 months	34.00^{f-z}	19.33^{r-z}	16.33^{t-z}	27.00^{j-z}	13.00^{w-z}	14.00^{u-z}	31.66^{i-z}	24.00^{m-z}	30.66^{h-z}
9 months	10.33^{yz}	24.33^{l-z}	15.00^{t-z}	16.00^{t-z}	18.66^{s-z}	20.66^{o-z}	22.66^{n-z}	12.33^{w-z}	16.00^{t-z}
12 months	15.33^{t-z}	28.33^{i-z}	17.00^{t-z}	7.33^{a-h}	6.66^{a-h}	5.00^{c-h}	4.00^{e-h}	8.00^{t-z}	21.33^{o-z}

*Means sharing similar letter(s) are non-significant at α = 5% (DMR test).

onto the fresh medium. Some genotypes had significantly better survival and regeneration percentages probably due to either high endogenous levels of growth regulators or tolerance to growth retarding compounds.

Conclusion

This study shows that the slow growth storage technique by reducing concentration of BAP in culture medium is useful to maintain pear genotypes for short term as duplicate storage, and also successful to extend subculture duration with minimum cost and least risk of genetic instability.

REFERENCES

Addicott FT, Lyon JL (1969). Physiology of abscisic acid and related substances. Ann. Rev. Plant Physiol. 20: 139-154.

Ahmed M, Anjum MA (2010). *In vitro* storage of some pear genotypes with the minimal growth technique. Turk. J. Agric. For. 34: 25-32.

Ahmed M, Anjum MA, Shah AH, Hamid A (2010). *In vitro* preservation of *Pyrus* germplasm with minimal growth using different temperature regimes. Pak. J. Bot. 42: 1639-1650.

Endtmann KJ (1999). Taxonomy and nature conservation of wild pear (*Pyrus pyraster*) and its congeneric taxa. Beitrage Forst. Landschaft. 33: 123-131.

Epperson JE, Pachioo DH, Guevera CL (1997). A cost analysis of maintaining cassava plant genetic resources. Crop Sci. 37: 1641-1649.

Fay MF (1994). In what situation is *in vitro* culture appropriate to plant conservation? Biodiver. Conserv. 3: 176-183.

Fowler C, Mooney P (1990). Shattering Food, Politics and the Loss of Genetic Diversity. The Univ. of Arizona Press, Tucson, USA.

Gunning J, Lagerstedt HB (1985). Long term storage techniques for *in vitro* plant germplasm. Proc. Intl. Plant Propag. Soc., pp. 199-205.

Kovalchuk I, Lyudvikova Y, Volgina M, Reed BM (2009). Medium, container and genotype all influence *in vitro* apple germplasm. Plant Cell Tiss. Org. Cult. 96: 127-136.

Moriguchi T (1995). Cryopreservations and minimum growth storage of pear (*Pyrus* species). In: Bajaj, Y.P.S. (ed.). Biotechnology in Agriculture and Forestry, 32. Springer Verlag, Berlin, Heidelberg, pp. 114-128.

Moriguchi T, Kozaki I, Yamaki S, Sanada T (1990). Low temperature storage of pear shoots *in vitro*. Bull. Fruit. Tree. Res. Sta. 17: 11-18.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Negri V, Tosti N, Standardi A (2000). Slow growth storage of single node shoots of apple genotypes. Plant Cell Tiss. Org. Cult. 62: 159-162.

Paprstein F, Kloutvor J, Holubec V (2002). Mapping of the regional cultivars of fruit woody species in the Czech Republic. In: Swiecicki WB, Naganowska, Wolko B (eds.). Broad Variation and Precise Characterization – Limitation for the Future. Proc. XVIth EUCARPIA Genetic Res. Soc. Workshop, 16-20 May 2001, Poznan, Poland, pp. 71-76.

Rao NK (2004). Plant genetic resources: Advancing conservation and use through biotechnology. Afr. J. Biotechnol. 3: 136-145.

Reed BM (1992). Cold storage of strawberries *in vitro*: A comparison of three storage systems. Fruit Var. J. 46: 93-102.

Scowcroft WR (1984). Genetic variability in tissue culture: Impact on germplasm conservation and utilization. IBPGR Technical Report, International Board of Plant Genetic Resources, Rome, Italy, p. 41.

Sĭndelar J (2002). Toward a threatened forest tree species preservation on the example of crab apple (*Malus sylvestris* L.) and wild pear (*Pyrus pyraster* L. [Burgsdorf]). Zpravy Lesnickeho Vyzkumu. 47: 199-203.

Wagner I (1999). Conservation and yield of wild fruit trees: Problems regarding direct uses of relics of wild fruit trees. Forestarchiv. 70: 23-27.

Wanas WH, Callow JA, Withers LA (1986). Growth limitation for the conservation of pear genotypes. In: Withers LA, Alderson PG (eds.). Plant Tissue Culture and its Agricultural Applications. Butterworth, London, pp. 285-290.

Westcott RJ (1981). Tissue culture storage of potato germplasm: Minimal growth storage. Potato Res. 24: 331-342.

Westcott RJ, Henshaw GG, Roca WM (1977). Tissue culture storage of potato germplasm: Culture initiation and plant regeneration. Plant Sci. Lett. 9: 309-315.

Whigham DF, Dykyjova D, Hejny S (1993). Wetlands of World. 1: Inventory Ecology and Management. Handbook of Vegetation Science. Kluwer Academic Publishers, London, pp. 768.

Wilkins CP, Cabrera PJL, Dodds JH (1985). Tissue culture propagation of trees. Outlook Agric. 14: 2-13.

Wilkins CP, Dodds JH (1983). Tissue culture propagation of temperate fruit trees. In: Dodds JH (ed.). Tissue Culture of Trees. Croom Helm, London, pp. 113-136.

Wilkins CP, Dodds JH, Newbury HJ (1988). Tissue culture conservation of fruit trees. FAO/Intl. Board Plant Genetic Resour. Newslett. 73/74: 9-20.

Withers LA, Alderson PG (1986). Plant tissue culture and its agricultural applications. Butterworths, London, p. 526.

Wunsch A, Hormaza JI (2007). Characterisation of variability and genetic similarity of European pear using microsatellite loci developed in apple. Sci. Hortic. 173: 37-43.